2. (Amended) The method according to claim 1 for differentiating embryonic stem cells to cells with markers characteristic of neural cells comprising:
(a) culturing the embryonic stem cells in the serum free and feeder-layer free media at low cell density wherein said density is selected to minimize ES cell aggregation or EB formation; and
(b) allowing said cells to differentiate.

a1 cont

3. (Amended) The method of claim 2 wherein the cell density is selected as to avoid EB formation.

a2

9. (Amended) The method of claim 7 wherein the differentiating ES cells form at least one sphere colony.

10. (Amended) The method of claim 1 wherein the differentiating ES cells form at least one sphere colony.

a3

13. (Amended) The method of claim 12 wherein the primitive neural stem cells are pluripotent.

14. (Amended) The method of claims 1 or 12 wherein the serum free media further comprises a growth factor.

a4

17. (Amended) The method according to claim 1 wherein the media comprises an inhibitor of TGF-ß superfamily signal transduction.

a5

19. (Amended) The method of claim 17 wherein the inhibitor is selected from the Cerberus family of proteins.

20. (Amended) A method for producing secondary primitive neural stem cell colonies comprising:
(a) culturing ES cells in low cell density serum-free and feeder-layer free media for a time and under conditions sufficient to differentiate the said ES cells to primary primitive neural stem cell colonies;
(b) dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and
(c) administering a growth factor or survival factor to the dissociated neural cells to produce secondary primitive neural stem cell colonies.

a6

25. (Amended) An isolated primitive neural stem cell expressing one or more neural precursor cell marker and/or one or more neural-specific mRNA molecule, and having multilineage potential.

a7

28. (Amended) A method according to any one of claims 1 or 12 for analyzing the role of genes in the regulation of neural fate specification.

29. (Amended) An isolated primitive neural stem cell produced by the method of claim 12 that comprises neural cell markers and is pluripotent.

30. (Amended) An isolated primitive neural stem cell.

33. (Amended) A method for screening for modulators of primitive neural stem cell differentiation comprising:
(a) culturing primitive neural stem cells in serum-free and feeder-layer free media under low density conditions in the presence of the potential modulator; under conditions that produce differentiation in the absence of the modulator;
(b) detecting any differentiation of the cells and cell types generated, if any;
(c) determining whether the modulator affects the differentiation of the cells.

36. (Amended) A method of claim 35 for screening for differentiation factors of neural cell development.

37. (Amended) A method for screening for differentiation factors of cellular development comprising:
(a) culturing the cells of claim 29 in serum free media, in the presence of the differentiation factor.
(b) detecting any differentiation of the cells.

Please re-number claims 38-45 to claims 39-46 and amend the same as follows:

39. (Amended) An isolated modulator or differentiation factor detected by the methods of claims 33-37.

40. (Amended) A method according to claim 38 for modulating cellular differentiation.

41. (Amended) The method of claim 1 for obtaining a homogenous uniform cell base.

42. (Amended) The method of claim 40 wherein the cell base is a neural cell base.

43. (Amended) A method for supplying cells for transplantation comprising culturing cells pursuant to the method of claim 1 or 12.

44. (Amended) A method for treating neurodegenerative disorders comprising administering to a patient in need thereof the cells of claim 29.

45. (Amended) A method for the treatment of any disease or conditions resulting from cell loss or function in the neural system comprising administering the cells of claim 29 to a patient in need thereof.

A10 cont

46. (Amended) A method of gene therapy, wherein the cell of claim 29 is modified to express a gene of interest and administering said modified cell to a patient in need thereof.

Please add new claims 47-50 as follows:

47. (New) A method for producing secondary primitive neural stem cell colonies comprising:

(a) culturing ES cells in low cell density serum-free and feeder-layer free media for a time and under conditions sufficient to differentiate the said ES cells to primary primitive neural stem cell colonies;

(b) dissociating and subcloning the primary primitive neural stem cell colonies generated from the said ES cells; and

(c) administering LIF or B27 to the dissociated neural cells to produce secondary primitive neural stem cell colonies.

A11

48. (New) The primitive neural stem cell of claim 30, wherein the cell is isolated from an embryonic stem cell.

49. (New) An isolated sphere colony comprising primitive neural stem cells.

50. (New) A method for modulating primitive neural stem cell differentiation comprising administering to a primitive neural stem cell a modulator of claim 39.

In the Specification:

Please replace the paragraphs from page 13, line 33 to page 15, line 20 with the following:

A12

--The derivation of neural cells (among other cell types) from EB derived cells in vitro has been previously documented (Doetschman et al., 1985). Several studies have shown that the differentiation of neurons and glial precursors from EB derived cells can be enriched in the presence of retinoic acid (Bain et al., 1995; Fraichad et al., 1995; Strubing et al., 1995), FGF2 (Okabe et al., 1996), or PDGF (Brustle et al., 1999). Also, BMP4 has been shown to suppress neuronal differentiation of EB derived cells (Finley et al., 1999). Although these observations clearly demonstrate the potency of such factors to promote or attenuate neuronal differentiation of ES cells, each experiment was preceded by EB